THE PHYSICAL ENVIRONMENT OF MICRO-ORGANISMS IN SOIL[1]

A. D. McLAREN AND J. SKUJINS

College of Agriculture, University of California, Berkeley

INTRODUCTION

For the present purpose we define soil as the loose surface layer of earth which supports the various organisms dwelling within. The soil is formed by various physical, chemical, climatic and biological factors and may be represented as a matrix of colloidal systems. The inert solid particles are complexes of minerals and organic polymeric substances

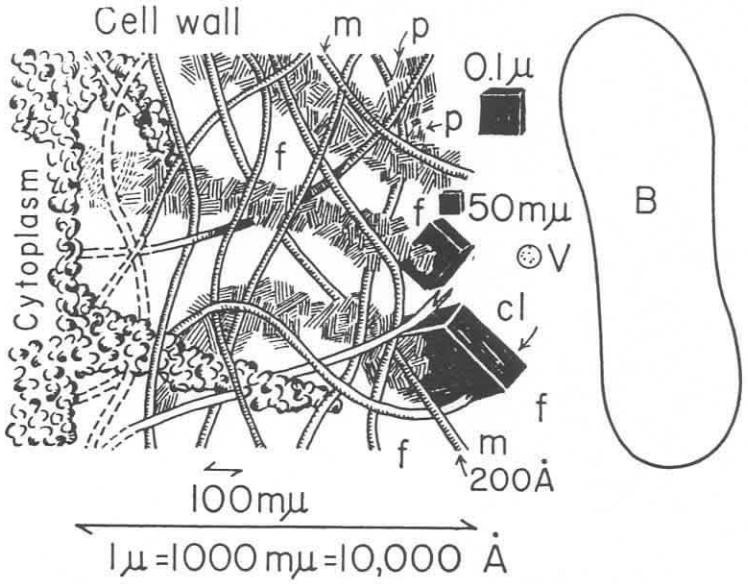

Fig. 1. Micro-environments of soil-plant root-microbe relationships. Modified figure from Lopez-Gonzales, J. de D. and Jenny, H. (1958). Modes of entry of strontium into plant roots. *Science*, **128**, 90. (m) cellulose microfibrils; (p) pectic substances; (f) free space; (Cl) clay particles of various sizes; (v) virus particle; (B) bacterium. The molecular environments are made up of surface-soil solution concentration gradients one order of magnitude smaller than illustrated (McLaren and Babcock, 1959).

(humus), in which are dispersed organic molecules, inorganic salts and ions, water and gases. Within the soil matrix are found micro- and macro-organisms in vast numbers and of many kinds, together with dead roots and excrements of soil fauna in all stages of disintegration

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(Fig. 1). Since micro-organisms may number 10^9 per gram of soil in a typical field soil, the soil may be regarded as a living tissue (Quastel, 1946). However, the physiological and biochemical activities of micro-organisms in such a heterogeneous system must differ drastically from those in classical bacteriological media. In soil there is a point-to-point variation in concentration of all solutes and further, the microbe may usually be thought to be on the verge of starvation.

It is not the purpose of this discussion to describe the many ecological niches of the micro-organisms resulting from the various chemical and nutritional aspects of the soil environment, but rather to consider some of the conditions pertaining in the soil environment which affect biological processes in soil. Incidentally, the physiological and biochemical relationships and activities of micro-organisms in soil environments have been reviewed recently (Alexander, 1964).

THE MICRO-ENVIRONMENT

Since there is a point-to-point variation in the concentration of solutes and gases in a soil matrix we may speak of soil *micro-environments*. These micro-environments consist of volume elements which are commensurate in size with an organism in question. Furthermore, at the surface of a soil particle and at the surfaces of the cells of micro-organisms themselves there exists a *molecular* environment characterized by gradations in ion concentrations, including the pH and the reduction-oxidation potential.

Micro-environments in the soil are discussed in terms of the diverse ecological conditions available to the micro-organisms.

Soil porosity

All biological activities and interactions are dependent on water, which in turn is dependent in part on the soil structure, and especially on the soil porosity. The ultimate solid particles in soil tend to aggregate, which gives a soil a texture. Thus, soil may exist in a finely dispersed, dusty state or in the form of soil aggregates of various sizes. Physically the structure may be characterized by two interdependent systems—a labyrinth of interconnecting pores and a matrix of contiguous solid particles.

Depending on the composition of the mineral fraction of the soils, its organic matter characteristics and on various external factors, such as climate and vegetation, the soil may consist of aggregates from the submicroscopic size to 20 mm. and more in diameter. Theoretical con-

siderations show that in a cubic type geometrical packing of equal size spheres the pore space is close to 50 per cent of the total volume. Similarly, a hexagonal type packing gives a porosity of only about 25 per cent. However, soil is not packed in a geometrically homogeneous manner and it contains particles of various sizes and shapes. In view of what follows, however, it is instructive to subdivide soil porosity into intra-aggregate and inter-aggregate porosity. In the intra-aggregate pores, gas concentrations can differ drastically from that between soil particles. In a permanent pasture the percentage pore space between particles was found to be c.15 (in a young ley it was only one-fourth as much) (Gadgil, 1963). Total pore space may be as high as 30–45 per cent.

Soil water

Examination of the physical structure of soil shows that water exists in the soil in several forms with respect to its retention. The soil-water relationship with respect to biological activities in soil and plant growth has been lately reviewed by several authors (Black, 1957; Marshall, 1959; Russell, 1961; Krasilnikov, 1961).

Gravitational water moves downwards in the soil under the pull of gravitational forces until it reaches subsoil or is retained in soil by forces counteracting gravitation. The ability of soil to drain water is called soil permeability. Gravitational water is available to soil organisms and it plays a major role in the transport of solutes in soil.

The capillary water moves in soil by means of capillary forces in the soil pores. The smaller the diameter of a pore the higher the water rises against the gravitational force. Pores in soil are of various dimensions and shapes and the soil structure greatly influences the water movements. In soils with large aggregates, water penetrates easily, whereas in soils without aggregates the pores are small and the water moves through capillaries slowly but it can rise quite high. Keen (1922), for example, has calculated that in a fine silt (0·01–0·002 mm.), water can rise to a theoretical height of 45 metres. The upward movement of the capillary water is enhanced by the surface evaporation. The capillary water is available to organisms and it also transports soil solutes.

Following removal of capillary water from soil, some water remains on and in the soil particles. This water is held to the clays by osmotic reduction of vapour pressure, by adsorption and by solution in polymeric humus. Part of this water may move as a liquid of higher viscosity and lower freezing point than bulk water. According to Low (1961), who has examined extensively the properties of this osmotically held

water at clay surfaces, the viscosity is increased at distances up to 60 to 100 Å from clay surfaces, depending on the clay type. This water comprises about 1 per cent of sandy soils and reaches about 10 per cent in humus-rich soils. According to data collected by Low (1961), it freezes at temperatures of −7 to −17°; such water held by quartz and montmorillonite freezes even below −25°.

The layer of adsorbed water, corresponding to a film up to perhaps 15 Å in thickness remains unfrozen at very low temperatures. Such tightly bound hygroscopic water can be mostly removed from soil by heating to 105°. All such bound water can be in equilibrium with water vapour over a range of relative humidities extending down to 1 to 5 per cent, which is far below the range of biological interest.

In biological studies the moisture regime has often been described in terms of moisture content of soils; however, such data give us little information related to the ability of an organism to absorb and utilize soil water. In order to utilize water, a growing organism must expend the energy required to remove water from soil and this depends on the types and sizes of the soil particles, the characteristics of the aggregates (soil structure), and on the concentration of the ions in the water phase. This energy may be expressed logarithmically by the pF scale (Schofield, 1935); that is,

$$\mathrm{pF} = \log\left(\frac{RT}{Mg} \times \ln\frac{p}{p_0}\right)$$

where R = gas constant, T = absolute temperature, M = molecular weight of liquid, g = gravitational constant, and p/p_0 = relative vapour pressure. In order to remove water from a 1·5 μ diameter

TABLE 1

Approximate relation between water suction and relative humidity

Relative humidity	pF	Relative humidity	pF
99·9	3·10	85·0	5·36
99·5	3·84	80·0	5·50
99·0	4·16	75·0	5·62
98·0	4·45	70·0	5·71
97·0	4·63	65·0	5·78
95·0	4·86	50·0	6·00
90·0	5·17		

capillary, commensurate with the diameter of bacteria, a suction of one atmosphere is required. This is equivalent to 1000 cm. water (pF 3), and a relative humidity of 99·9 per cent (Table 1, Griffin, 1963). In other words, for the organism to obtain water from such a capillary, a free energy of about a half calorie per mole of water must be expended; the organism can easily compete with a plant root system. Conversely, plants show a permanent wilt at or below about 99 per cent relative humidity and it will be of interest to compare the extent to which some soil microbes can withstand drought.

Actually, the mechanisms by which the water is held in soil (capillary, osmotic and hygroscopic water) operate simultaneously. These mechanisms may be distinguished by the energy of the negative suction pressure that the water in any particular region exerts, e.g. gravitational water (not a true water holding mechanism by a strict definition), 0 to 0·3 atmospheres; capillary water, 0·3 to about 15 atmospheres; osmotic water, approximately 15 to 150 atmospheres (this value is strongly influenced by the solutes present); hygroscopic water, above 150 atmospheres. For a comparison, it may be noted that 143 atmospheres of water suction is equivalent to 90 per cent relative humidity.

In the studies of biological activity in drying and remoistened soils, it should be noted that the soils exhibit a so-called hysteresis phenomenon. If a wet soil is dried at a certain relative humidity A, the soil will equilibrate with a certain soil moisture content B_1; however, if the soil is dried further and then subjected to the relative humidity A, the equilibrated soil moisture content B_2 will be less than B_1. In some soils, the difference in moisture content might be considerable, depending on the treatment (Schofield, 1935).

Microbial response to soil water content

Generally, the additive effects of tensiometer and osmotic components of soil water have been taken to constitute the total stress against which an organism must do work to absorb water (Richards and Wadleigh, 1952); however, other hydraulic factors also appear to be important (Gingrich and Russell, 1957). The bacterial activity in soil with respect to moisture content has been widely studied on an empirical level, and generally, a good correlation has been found; microbial activity increases with increasing water content to about 60 to 80 per cent of the water-holding capacity and decreases in waterlogged soils (Seifert, 1960, 1961; Gondo, 1961).

The maximum activities of micro-organisms may depend on the thickness of the water film surrounding the solid soil particles. Rahn

(1913), found the optimum thickness of the film to be 20 to 40 μ for *Bacillus mycoides*; this corresponds to a pF of a little less than 2. Some soil micro-organisms may develop at relative humidities down to 85 per cent or less. Dommergues (1959) studied microbial activities at low moisture levels and showed that the minimal moisture content in soil for cellulolytic organisms was 1·5 per cent and glucose-utilizing organisms 0·7 per cent, which was considerably below the wilting point (1·7 per cent). Cell division of *Pleurococcus vulgaris* occurs at a relative humidity of 48 per cent at 20° (Zeuch, 1934). This is extreme: at this relative humidity a typical protein contains only about 8 per cent water, at a partial molar-free energy of about 500 calories (McLaren and Rowen, 1951). If the cell is also at such a low water content, enzyme reactions must take place in the absence of a liquid water phase and at very low velocities (Ruchti and McLaren, 1964).

Available data for the minimal relative humidity requirements for growth of soil fungi have been collected by Griffin (1963). Minima for most of the fungi lie between 85 and 95 per cent relative humidity, but *Aspergillus amstelodami*, *A. glaucus* and *A. repens* have minimal relative humidity requirements of 70 to 72 per cent and *Penicillium glaucum* 78 per cent. For most fungi, however, growth decreases by half at 94 to 97 per cent, as compared with growth at 100 per cent relative humidity.

Excellent survival of some micro-organisms in air-dry soil is a well-known phenomenon. The maintenance of stock microbiological cultures in dried soil suspensions is an accepted method for preserving bacteria. Non spore-formers, such as *Nitrosomonas*, may be recovered from air-dry soils after several years. Preservation of these organisms on other bacteriological maintenance media for the same period of time would fail.

A study of the survival of some 50 fungal isolates in five-year-old dried soil cultures showed a wide range of ability to survive. *Rhizopus nigricans*, for example, showed an extremely high survival capacity, whereas *Fusarium oxysporum* showed the least viability (Atkinson, 1954). Studies on survival of algae in Rothamsted soils, kept hermetically sealed in an air-dry state for 26 to 73 years, showed that a number of Cyanophyceae, Chlorophyceae and one species of diatom could be recovered (Bristol, 1919). We have shown that after 10 years of storage, however, the general population of soil micro-organisms decrease about 100-fold, i.e. from about 10^{-7} organisms to 10^{-5} organisms per gram of soil (unpublished results). Nevertheless, the survival of the remaining organisms may be due to the osmotic and hygroscopic water present in the soils stored air-dry.

Soil solution

The water phase in soil carries dissolved compounds of various types and forms: inorganic ions, including the nutrient minerals for micro-organisms, and organic compounds—the metabolic products of the living organisms in soil. It has been shown that the soil solution contains also dispersed colloidal material, comprising 5 to 20 per cent of the dry weight of the solution residue; the major constituents of the colloidal fraction are of organic nature, but there are also iron and aluminium hydroxides and silicic acid.

The amounts and ratios of the compounds in the soil solution fluctuate with respect to the characteristics of the soil type and the climate. Upon drying the salt concentration increases and the salts precipitate according to their solubilities.

The soil solution is the liquid nutrient medium for the soil micro-organisms. In connection with Quastel's notion of the soil as a living tissue, it is interesting to note that already in 1902 Visotskii had compared the soil solution to the blood of animals. The nutrients found in the extracted soil solutions of specific soils greatly influence the make-up of microflora found in any particular soil (Krasilnikov, 1961).

Decrease of soil moisture not only limits the available water to micro-organisms, but also increases the salt content in the soil solution. In solute-rich soils specific halophytic populations become established, but the effects of an increase in ionic concentrations due to soil drying on micro-organisms in regular soils has been little studied. Johnson and Guenzi (1963), have pointed out that increasing osmotic tension with increasing salt concentration causes a decrease in nitrifying activity, but the total microbial activity is less affected, and that microbial populations show greater salt tolerance in calcareous than in noncalcareous soils. It should be noted that many individual species of bacteria can tolerate relatively large changes in the salt concentration.

Soil atmosphere

The pore space in soil is either filled with air, as in dried soils, or filled with water, as in waterlogged soils. In the first case all life processes practically cease, in the latter case anaerobic processes predominate. The air-water ratio in the pore space is of utmost importance for determining the types of biological processes in soil. Most of the soil micro-organisms require a certain air-water ratio for their maximal proliferation as already noted (Rahn, 1913).

Gases in soil exist in three states: (1) a free state, filling the otherwise

empty soil pores, (2) dissolved in the water phase and (3) adsorbed on the solid phase.

The adsorption of the atmospheric components by the solid soil matter is of importance only in very dry soils. With an increase of moisture the sites for hygroscopic adsorption of water become occupied, and generally, the water molecules are adsorbed by the soil particles more tenaciously than gases.

The solubility of gases in the water phase depends on the type of gas, temperature, the salt concentration in the water phase, and on their partial pressures in the atmosphere. The most soluble are gases which become ionized in water solution, as CO_2, NH_3 and H_2S. Oxygen is less soluble (0·31 cm.^{3} per litre of water at 10°, 760 mm. Hg.), and nitrogen the least soluble (0·15 cm.^{3} per litre at 10°, 760 mm. Hg.). Because of dissolved electrolytes the solubility of gases in the soil water is less than in pure water. The total amount of air in soil fluctuates widely and it is dependent on the soil porosity and moisture content, as well as on microbial and root activity. Penman (1940*a*, 1940*b*), and Currie (1962), among others have discussed the problem of gas diffusion in soil. Generally, oxygen continuously diffuses into the soil and CO_2 diffuses out; for porosities greater than 10 per cent by volume of soil the rate of exchange of oxygen and carbon dioxide with the surface atmosphere comes to equilibrium down to a depth of 30 cm. in about an hour. The ratio of CO_2/O_2 increases with increasing depth and with a decrease in the available porosity. The sum of the partial pressures of CO_2 and O_2 remains nearly constant at 21 per cent of the total pressure. When the water content in soil is increased and the available air pore volume is less than 10 per cent, the above relationship does not apply, the physical shape of the pores becomes more important for diffusion rates, and eventually O_2 concentration approaches zero in waterlogged soils.

It should be emphasized that the equilibrium conditions between water—water vapour—oxygen and CO_2 in the soil pores are greatly influenced by temperature fluctuations, including the diurnal changes. The effects of temperature changes on the equilibria in the soil liquid and gaseous phases with respect to soil organisms have been described by Collis-George (1959).

Generally, the soil atmosphere differs from the surface atmosphere by its higher CO_2 content, which is formed at the expense of O_2. Nitrogen is almost constant. Besides the atmospheric gases, there are also metabolically formed gases, e.g. NH_3, CH_4, H_2S, and also many biologically formed volatiles, e.g. organic acids, esters, hydrocarbons, etc.

Although nitrogen is of prime importance for nitrogen-fixing orga-

nisms, the nitrogen availability for microbes in soil has been but little studied (Shields and Durrell, 1964).

It has been suggested that the regime of CO_2 in soil is important not only because it affects the metabolism of autotrophic organisms and influences the pH at the microsites in soil, but also because of its potential role as a differential inhibitor for heterotrophs (Alexander, 1964). Papavizas and Davey (1962) have shown that the saprophytic growth of *Rhizoctonia* in soil was reduced at 10 to 20 per cent CO_2 concentration and drastically inhibited at 30 per cent concentration. Blair (1943) found that forced aeration of the soil increased the growth rate of *Rhizoctonia solani* and that the growth rate was also increased when CO_2 from soil was absorbed by chemical means. Newcombe (1960) reported that under an atmosphere of CO_2 conidia of *Fusarium oxysporum* germinated freely but failed to form chlamydospores.

The various combined effects of soil structure, water availability and aeration of fungal growth was critically examined and reviewed by Griffin (1963) who used artificial soil (aluminium oxide grits) of known pore size. Growth was inhibited in waterlogged conditions, but normal growth appeared if sufficient suction was applied to drain pores within limits of 16 to 550 μ diameters, irrespective of particle size. The sporulation did not seem to be affected by the suctions used, but its intensity was greatly reduced when the pore diameter was reduced less than 23 μ.

Scott and Evans (1955) have shown that oxygen rapidly disappears not only in completely waterlogged soils but also in well-structured and aerated soils under conditions where waterlogged crumbs are present. Winogradsky (1924) determined depths to which an aerobe, *Azotobacter*, and an anaerobe, *Clostridium*, could grow in wet soils; at a moisture content of 23 per cent the former was limited to the extreme surface and the latter was found throughout the soil. In view of the high concentration of CO_2 found in soil after rain (3·5 to 9·2 per cent), CO_2 content rather than low O_2 content may determine the vertical distribution of soil fungi (Burges and Fenton, 1953).

Recently, from a study of widely different soils, it has become evident that the changeover from aerobic to anaerobic metabolism takes place at an oxygen concentration of less than about 3×10^{-6}M (Greenwood, 1961; Greenwood and Berry, 1962). It appears that water-saturated soil crumbs larger than about 3 mm. in radius have no oxygen at their centres and, since crumbs of this size are present in most soils, it means that microsites having anaerobic conditions are ubiquitous, and this provides an explanation for the universality of strict anaerobes. Oxidative processes, however, abound in relatively warm, well-aerated

soils. In relatively wet soils organic matter content increases with increasing rainfall (Jenny, Bingham and Padilla-Saravia, 1948).

A delay in nitrification processes, due to anaerobic metabolism taking place in soil, has been observed (Brandt, Wolcott and Erickson, 1964). The rate of nitrification is enhanced if the crumb diameters are below 1 mm. (Seifert, 1962) and an improved soil crumb dispersion with soil conditioners can increase the rate of nitrification. A similar study (Hagin, 1955) showed that in soils where aggregates were stabilized with a soil conditioner, the nitrification rate was doubled at the crumb size range of 0·5 to 5 mm. diameter, whereas ammonia accumulation occurred in soils with crumb sizes $< 0{\cdot}5$ mm. in diameter.

The existence of appreciable differences in population sizes at microsites within the soil was demonstrated by the study of Tyagnii-Ryadno (1958), who showed that considerably larger numbers of bacteria proliferate on the crumb surfaces as compared with the interior of aggregates.

The lack of oxygen in anaerobic (waterlogged) conditions is not the only parameter determining the type of microflora. With anaerobic conditions several chemical changes can take place which potentially influence the composition of the microflora. These include increases in soluble Ca, Mg, Fe, Ni, Mn, and Co compounds, accumulation of Fe^{++}, and decrease of NO_3^- and of available phosphorus. Fatty acids and other oxidizable organic compounds accumulate in this reduced state, and the reduction-oxidation potential decreases (Siuta, 1962; Ng and Bloomfield, 1962; Grechin, 1963).

Soluble plant and animal residues and products of microbial metabolism may be translocated and adsorbed on the soil particles in micropores physically inaccessible to micro-organisms. Rovira and Graecen (1957) showed that the decomposition of soil organic matter was accelerated when the micro-pores of the aggregates in certain soil types were disrupted. A burst of microbial activity in freshly moistened air-dried soils is a well-known phenomenon and in part such an increase in activity has been ascribed to the effect of drying on the organic matter which renders some of the organic matter more soluble (Griffiths and Birch, 1961). However, a disruption of aggregates upon drying, thereby making the organic substrates accessible to micro-organisms when rewetted, may also be a contributing factor.

The oxygen concentration directly affects the red-ox potential (E_h); in the absence of oxygen the negativity of the soil increases. Environmental limits of E_h (correlated with pH) for a large number of different micro-organisms in natural environments have been established by Baas-Becking, Kaplan and Moore (1960). However, it is not yet clear

whether the red-ox potential *per se* is important for microbial activity or if it is only a reflection of the oxygen availability and the influence of reducing or oxidizing compounds on micro-organisms.

It is of interest to note that the soil organic matter, especially the soil humic acids, contain stable-free radicals in a range of about 10^{18} radicals per gram of humic acid (Steelink and Tollin, 1962; Tollin, Reid and Steelink, 1963). The free radicals appear to be an integral part of the humic acid molecular structure. They may consist of a mixture of semi-quinone-type radicals and electron-transfer complexes of quinhydrone type. Any influence of free radicals on microbial growth in soil is unknown.

THE MOLECULAR ENVIRONMENT

As mentioned earlier, micro-organisms live in a complex soil matrix, where the solid phase is characterized by discrete solid particles having a spectrum of size. A full understanding of microbial behaviour in the micro-environment requires an examination of certain colloidal properties of the organic and inorganic solids. These properties include adsorption of solutes and exchange of cations and anions, Donnan equilibrium behaviour and others. Considering the scope of the subject-matter, only some topics of importance for microbial biology will be considered here; the reader's attention is directed to several excellent review articles regarding the physical chemistry of soil, namely by Toth (1964), Wiklander (1964) and Babcock (1963), among others.

Effect of electrokinetic charges at interfaces

The colloidal fraction of soil is made up of inorganic crystalline clay particles, organic decomposed residues, organic matter-clay complexes and organic macromolecules. Any of the colloidal particles, especially the clay particles, may be coated with iron, aluminium, or manganese hydroxide gels. These colloidal particles in soil have electric charges which are of prime importance for nutrient cation and anion retention and availability. Soil colloids have negative charges. The clay particles are very strongly electro-negative; organic soil colloids and the hydroxide-coated colloids are more or less amphoteric. In an aqueous phase the negative charge requires a cloud of cations for an overall state of neutrality. This cloud includes the biologically important H_3O^+ ion.

Hartley and Roe (1940) derived an equation for the difference between

the pH at a charged interface, pH_s, and that of an adjacent bulk phase, pH_b, by an application of the theory of Debye and Hückel. They pointed out that the electrokinetic potential (zeta potential, ζ) can be identified with the potential ψ in the neighbourhood of a simple ion at the distance of the closest approach of another ion, as considered by Debye and Hückel. In this sense the zeta potential determines the local concentration of ions near the surface of a particle, and the hydrogen ion concentration near the surface will be $e^{-\varepsilon\zeta/kT}$ times the H^+ concentration in bulk. The effective dissociation constant becomes

$$K_s + K_b e^{-\varepsilon\zeta/kT} = K_b e^{-F\zeta/RT}$$

where K_b = thermodynamic dissociation constant in bulk, ε = electronic charge, F = faraday, T = absolute temperature, and k = Boltzmann constant.

At 25° this equation may be rewritten as

$$pK_s = pK_b - \zeta/60$$

or

$$pH_s = pH_b + \zeta/60$$

where pH is in terms of H^+ concentration, not activities. No distinction between concentration and activity of H^+ is needed in bulk under physiological conditions near neutrality. That an enzyme acting at a charged surface responds to hydrogen ion concentration rather than activity has been shown elsewhere (McLaren and Babcock, 1959). The last equation can be evaluated via electrophoretic measurements by substituting, for large particles, the Smoluchowski relationship

$$\mu = \frac{\zeta D}{4\pi\eta}$$

We thereby arrive at the experimentally useful equation

$$pH_s = pH_b + 0{\cdot}217\ \mu$$

For small particles, Hartley and Roe give at 25°,

$$pH_s = pH_b + 0{\cdot}325\ \mu$$

The mobility of a particle μ is reckoned negative for motion toward an anode, in microns/sec./volt/cm.; D is the dielectric constant and η is the viscosity of the disperse medium at the same temperature. The

effect of the zeta potential in practice extends from the interface of the colloidal particle to 50–150 Å into the liquid phase. Several consequences of the existence of Δ pH (equal to $pH_b - pH_s$), in biological systems have been further considered by McLaren (1960). Here we concern ourselves with soil enzymes and bacteria.

A wide variety of enzymatic activities exist in soil, some of which has been attributed to extracellular enzymes (Skujins, 1966). Although the precise physical state of the extracellular enzymes in soil is poorly understood, it is apparent that some of the enzyme protein molecules are adsorbed on surfaces of the colloidal soil particles; they may also exist in some type of covalently bound form with the organic macromolecular components. Incidentally, catalytically active enzyme derivatives, covalently bound to organic polyelectrolyte copolymers, have been prepared by Riesel and Katchalski (1964) and Levin *et al.* (1964).

In studies with adsorbed chymotrypsin (McLaren and Estermann, 1957), phosphatase (McLaren and Ramirez-Martinez, unpublished results) and urease (Durand, 1964*a*) on clay particles, or with the trypsin-polyelectrolyte copolymer (Goldstein, Levin and Katchalski, 1964), it is clear that because of the ionic double-layer surrounding the clay particles, the observed pH maxima of the respective enzymatic activities were several units higher than in liquid solutions.

In context with the foregoing concepts the micro-organisms themselves should be considered as colloidal soil particles exerting their own field of zeta potential around the cells. Attempts to explain the apparent pH activity shifts of bacteria adsorbed on soil particles solely on the basis of the H^+ ion concentration in the double-layers of the soil particles have given conflicting results. Doubtless, the interplay of electrokinetic potentials of the adsorbing clay particle, and that of the bacterial cell should be considered, i.e. a cloud of a higher H^+ concentration exists not only around the clay particle, but also around the microbial cell of a distinct but different magnitude. Also, the distinction between the adsorbed and the adsorbing particles becomes semantic. Lahav (1962), for example, has studied the adsorption of small clay particles by bacterial cells.

Nitrification in soil takes place at a hydrogen ion concentration as high as pH 4. However, in solutions there is no nitrification below pH 6 (Weber and Gainey, 1962). McLaren and Skujins (1963) have shown that optimum nitrification by organisms in soil and adsorbed on cation exchange resins, irrespective of surface change, takes place at a pH greater than in solution.

Hattori and Furusaka (1960) have studied the biochemical activities

of *Escherichia coli* and of *Azotobacter agilis* (Hattori and Furusaka, 1961) adsorbed on an anion exchange resin. In both cases the peak activity of the adsorbed cells was at a pH greater than that of the cells in suspension.

To resolve the question of the effect of ionic environment at the molecular level on the activities of micro-organisms more experimental data are needed. It should be emphasized that not only the H^+ and OH^- equilibrium around the charged particles is of importance, but that those ions may be replaced by others, including anionic and cationic species of the bacterial substrates. These other ions may be present in quite an excess over the H^+/OH^- species and exert an inhibitory or stimulatory effect on the micro-organisms. In these cases, correlations with the pH might be only coincidental.

The acidity (pH_b) of soils has been very extensively studied and very good empirical correlations with microbial activities, ecology and pH have been found. Such information, however, does not tell us very much about the influence of the hydrogen ion concentration on the bacterial cell within its micro-environment. The effects of the anionic part of an ionized acid molecule on micro-organisms has been studied in general bacteriology, but there is no information relating these effects to the soil microflora (Eno, 1957). Obviously, H^+ exerts a direct effect on a bacterial cell, but at the same time, changes in the hydrogen ion concentration in the soil markedly change the availability of various inorganic and organic nutrients. Again, only meagre data exist in the literature pertaining to the separation of these two hydrogen ion effects.

Sorbtive phenomena in soil

Typical colloidal particles in soil exhibit strong sorbtive properties. Several types of interactions are of importance in the microbial soil micro-environment, sorption of micro-organisms on soil particles, sorption of colloidal and monomolecular organic nutrients and microbial metabolic products on solid soil particles; and the activities of adsorbed extracellular enzymes at liquid-solid interfaces.

The adsorption of micro-organisms by soil and similar solid particles has been extensively studied in the 1920s and 1930s, as reviewed by Estermann and McLaren (1959). Some later studies have shed some light on the mechanisms involved in the adsorption processes. Tschapek and Garbosky (1950), made an extensive study of *Azotobacter* adsorption by surface-neutral quartz sand, and of the various factors affecting it. Zvyagintsev (1962), has presented an enlightening study of adsorption

of various soil micro-organisms by cation and anion exchange resins. Micro-organisms of various species were adsorbed on an anion exchange resin, especially at pH values below pH 6. There was no adsorption at an alkaline pH. Selective adsorption with respect to species occured on cation exchange resin and the ions saturating the resin exhibited a striking effect: adsorption took place if the cation exchange resin was saturated with divalent and trivalent ions or H_3O^+, but there was no adsorption on the resin saturated with monovalent ions. Thus, the adsorption was dependent on the initial surface charge on the solid particles, the cations present in the solution and on the pH, correlating nicely the adsorption phenomena with our present understanding of the surface charge effects of solid particles (including microbes), and with the double-layer theory.

It is interesting to note that Lahav studied adsorption of colloidal clay particles to *Bacillus subtilis* cells and noted the presence of two distinct cell types: the adsorbing and the non-adsorbing *B. subtilis* cells (Lahav, 1962). Zvyagintsev (1959) found that the adsorption of bacteria on glass surfaces is strongly dependent on the taxonomic characteristics of the organisms and that the adsorption is not an 'all or none' phenomena but varies in a range from none to very strong.

It has been rather difficult to establish and to distinguish the numbers, ratios and types of adsorbed and non-adsorbed bacteria in soils. A recent study of Aristovskaya (1963) indicates that the bacterial flora of the soil particles consists mainly of attaching and creeping species, the majority of which form micro-colonies. The non-adsorbed inhabitants of the soil solution are mostly individual, single-cell organisms. The adsorption of viruses by soil particles was noted by Miyamoto (1959), among others.

Although it is generally recognized that soil particles might adsorb a great variety of organic compounds, the literature contains rather meagre specific data in this respect. Apart from the important function of the adsorbed organic metabolites as nutrients for micro-organisms, adsorption of the organic matter by mineral particles might be the first step in the formation of the biologically resistant humus fractions.

The adsorption of small organic molecules by clay particles, such as antibiotics (Krasilnikov, 1961), purines and pyrimidines (Durand, 1964*b*) and amino acids (Putnam and Schmidt, 1959; Greenwood and Lees, 1960), is of interest; the adsorption of large colloidal polymers (proteins, carbohydrates, etc.) also must play a large role in soil processes.

Protein molecules, released in soil by microbes, soil fauna or plant roots, are polyelectrolytes; i.e. water-soluble, ionized polymers. The chemical and physical behaviour of these polymer molecules is determined by the composition of the polymer chain, ionization and van der Waal's forces. The adsorption of macromolecules on solid surfaces has been reviewed by Silberberg (1962*a*, 1962*b*), and by McLaren and Peterson (1965). Some examples of the influence of adsorption on soil metabolism are now discussed. Goldberg and Gainey (1955) reported that the availability of ammonia to nitrifying bacteria proliferating on different soil minerals was directly related to the quantity of unadsorbed ammonia or to the quantity of NH_4^+ released from the soil minerals by the cation exchange mechanism. Ammonium fixed by illite and bentonite is nitrified very slowly. Incidentally, the process of nitrification is further reduced by additional increase in K^+ (Welch and Scott, 1960).

It appears that about 35 to 45 per cent of the total nitrogen in soil is in the form of polymers containing amino acid residues (Bremner 1949, 1955). Although the actual chemical composition of these polymers is unknown much of it may be adsorbed by clay particles. The adsorption of proteins on clays has been studied by Ensminger and Gieseking (1939, 1941) and by McLaren (1954*a*). Generally, proteins are adsorbed on clays in a wide pH range because of the amphoteric nature of the proteins and the relatively strongly, negatively charged clay particles. Proteolytic enzymes may be adsorbed on the clay-protein complexes and hydrolyse adsorbed proteins. An entirely new form of the chemical kinetic equations is required to handle enzyme reactions on insoluble substrates (McLaren, 1963). The enzymes may be desorbed from the complexes with a minimal loss of activity (McLaren, 1954*b*; McLaren and Estermann, 1956). Depending on the type of protein and on the experimental conditions, a single monolayer (McLaren, Peterson, and Barshad, 1958) or several layers (Talibudeen, 1950) of protein may be adsorbed on clays. Upon adsorption of protein the clays expand as the protein enters the interlayer space of the crystal lattices (McLaren, Peterson, and Barshad, 1958; Armstrong and Chesters, 1964).

Studies have shown that not only the protein adsorbed on the outside surfaces of clay particles but also the protein present in the interlayer space is utilized by micro-organisms, suggesting that extracellular proteolytic enzymes have access to the interlayer space (Estermann, Peterson and McLaren, 1959). Both stimulatory and retarding effects of adsorbents on the metabolic rates of bacteria have been observed (Estermann and McLaren, 1959). For example, *Bacillus subtilis* adsorbed on

clays respire slower than in the free state. Lahav and Keynan (1962), however, have shown that under certain conditions the respiration of *B. subtilis* is enhanced. Macura and Pavel (1959) demonstrated that the adsorption of *Azotobacter* on clays increased the rate of nitrogen fixation.

A study of the bentonite-adsorbed urease by Durand (1964*a*), mentioned above, showed that in the presence of bentonite, Cu^{++} was considerably less effective as an inhibitor. A very striking stimulation of uricase activity on uric acid was evident when the enzyme was adsorbed on bentonite (Durand, 1964*b*). On the other hand, another recent study on the retardation of the proteolytic activity in presence of clays (Aomine and Kobayashi, 1964) showed that the type of clay used for adsorption had a drastic influence on the activity: allophanic clays inhibited the protease activity to a much greater extent than montmorillonite or halloysitic clays.

A very striking and interesting interaction between clay minerals and soil fungi was examined by Stotzky and Martin (1963). The spread of the pathogenic *Fusarium oxysporum* f. *cubense* was completely correlated with the presence of a certain specific clay mineral in the soil. The mechanism of this clay-fungus interaction is not understood.

Apart from clays, other colloidal material in the soils, e.g. water-soluble constituents of plant residues (Handley, 1961), may significantly influence the availability of proteins to micro-organisms.

Although, with few exceptions, proteinases are almost the only enzymes which have been studied in relation to their activities on adsorbed substrates, it should be noted that other extracellular enzymes may act in the soil environment in an adsorbed state. In a study associated with the lysis of fungal walls by the enzymes of soil *Streptomyces* spp. (Skujins, Potgieter and Alexander, 1965), it was noted that the extracellular streptomycete chitinase was rapidly adsorbed by the fungal wall constituent chitin which is degraded (Skujins, unpublished data).

When considering enzyme reactions in soil, one should be aware of the changes that the molecular environment in the soil imposes on enzyme kinetics. Terms used in traditional enzyme chemistry, such as 'moles per litre' are meaningless in such structurally restricted systems (McLaren and Babcock, 1959) and equations using mole fractions instead of concentrations have been developed (McLaren, 1962). Enzymatic kinetics expressed in terms of mole fractions are useful in structures of low water content and reaction rates on surfaces and in gels may be meaningfully examined.

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